PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A23K 1/18, 1/165

A1

(11) International Publication Number: WO 91/04673

(43) International Publication Date: 18 April 1991 (18.04.91)

(21) International Application Number: PCT/DK90/00256

(22) International Filing Date: 5 October 1990 (05.10.90)

(30) Priority data:
4940/89 6 October 1989 (06.10.89) DK

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(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: FEED ADDITIVE FOR POULTRY, POULTRY FEED AND METHOD OF POULTRY FEEDING

(57) Abstract

The feed additive for poultry comprises a cellulase or a xylanase producible by means of selected strains of Humicola insolens and Trichoderma longibrachiatum. By means of this additive the malabsorption syndrome is effectively alleviated.

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FEED ADDITIVE FOR POULTRY, POULTRY FEED AND METHOD OF POULTRY FEEDING

The invention comprises a feed additive for poultry, a poultry feed, a method of poultry feeding, and a use of the feed additive for poultry. The feed additive is used as a medicament for alleviation of the malabsorption syndrome in broiler chickens.

Malabsorption syndrome is a problem observed in broilers in many countries of the world. This syndrome causes very important losses to the poultry industry. The affected flocks show several variable clinical symptoms:

- Lower digestion of feeds causing diarrhoea, particularly fats and fat soluble substances, or only wet litter,
- reduced fat digestability
- poor growth (runting or stunting syndrome)
- high feed:gain ratio
- brittle bones
- defective feathering
- defective pigmentation of the skin (pale bird syndrome): The yellow colour of affected birds is less than normal and the pigmentation is quite variable between birds of a same flock; this defective pigmentation is due to a bad utilization of the pigments called xanthophylls contained in feeds.

Sometimes only one of these symptoms exists in a flock. Sometimes several symptoms can be seen in the same flock.

The etiology of the syndrome is not well determined.

Several viruses have been cited as possible causal agents of the syndrome, e.g. Rotavirus, reovirus, coronavirus, adenovirus, calicivirus, parvovirus, and togavirus.

Mycotoxins have also been cited as possible causal agents of the syndrome.

The invention describes a feed additive for alleviation of the malabsorption syndrome in poultry, a poultry feed, and a method of poultry feeding.

The feed additive for alleviation of the malabsorption syndrome in poultry according to the invention is characterized by the fact that it comprises a cellulase and a xylanase producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma longibrachiatum* (NRRL 11460). NRRL 11460 was originally deposited as *Trichoderma reesei* and later reclassified as *Trichoderma longibrachiatum*. Surprisingly it has been found that this feed additive containing as the active component a specific cellulase/xylanase combination exhibits the ability effectively to alleviate the malabsorption syndrome in poultry.

Due to the fact that part of the malabsorption syndrome comprises a reduced fat digestibility, the obvious constituent of a feed additive for alleviation of the malabsorption syndrome would be a lipase. According to the invention, however, it surprisingly has been found that lipase does not alleviate the malabsorption syndrome to any significant degree. Even more surprisingly, as above indicated, it has been found, that the above indicated feed additive with the most specific cellulase/xylanase combination effectively alleviates the malabsorption syndrome, despite the fact that no corresponding theoretical explanation at present can be provided.

It belongs to the prior art that the growth of poultry can be improved by addition of cellulase and/or xylanase to the feed, vide e.g. SU patent no. 933.063 (referenced in Derwents abstract in World Patent Index Latest), and DK patent application no. 805/88, and selected cellulase/xylanase enzyme complexes for that purpose are described in SU patent no. 577.230 (referenced in Derwents abstract in World Patent Index), and DE 1.904.239. However, the feed:gain ratio of the prior art feeds are not satisfactory, and it has been found that the feed:gain ratio of the poultry feed according to the

invention is significantly better than the feed:gain ratio of the prior art poultry feeds, vide Example 6. More important however, according to the invention we are able to offer an alleviation of the symptoms of the malabsorption syndrome which is not possible according to the prior art. There is also the advantage that due to this improvement less manure has to be disposed of according to the invention, whereby less pollution is generated according to the invention.

The poultry feed according to the invention is characterized by the fact that the poultry feed is composed of a normal poultry feed, to which a feed additive according to the invention has been added.

The method of poultry feeding according to the invention is characterized by the fact that the poultry feed is the poultry feed according to the invention. Thus, the method according to the invention consists in administrating specific cellulase/xylanase enzyme combinations, by oral route, to birds raised in areas where the malabsorption syndrome exists endemicly.

A preferred embodiment of the method according to the invention is characterized by the fact that the feed additive according to the invention is added to the drinking water for the poultry.

Another preferred embodiment of the method according to the invention is characterized by the fact that the feed additive according to the invention as a solid material is added to the normal poultry feed.

It is known from the prior art that microbial enzymes may improve the growth of chickens when such enzymes are incorporated into feeds and the range of enzymes includes amylase, cellulase, hemicellulase, glucanase, lipase, gumase, chitinase and cytolytic enzymes. However, the specific combination of the enzymes producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma longibrachiatum* (NRRL 11460) is needed.

The present invention demonstrates how the addition of a selected cellulase/xylanase enzyme complex is able to alleviate the effects of malabsorption syndrome and return the performance of the chickens to their

normal state. It is also demonstrated that traditional cellulases do not have the same effect.

The examples given below demonstrate the properties and advantages of the invention.

SP 343 is the *Humicola insolens* cellulase/xylanase complex from DSM 1800, and SP 431 is the xylanase from *Trichoderma longibrachiatum* (NRRL 11460).

EXAMPLE 1

160 chicken were taken randomizedly, at 8 days of age, from a flock raised in an area where malabsorption syndrome was endemic.

These 160 chicken were afterwards raised in individual cages, on wired floor. Each cage was equipped with a feeder and a waterer in order to provide feed and water ad libitum.

The 160 chicken were divided in 4 lots of 40 birds. Each lot received one of these 4 experimental feeds:

1) Basal feed - wheat based (control)
2) Basal feed supplemented with enzyme SP 343 at 200 g/ton
3) Basal feed supplemented with enzyme SP 343 at 1000 g/ton
4) Basal feed supplemented with Lipozyme 10,000 L at 50 g/ton

After 8 days of experiment, feces were collected for each bird for 3 days. The collected feces of 2 birds consuming the same feed were pooled together: Results of the nutritional balance trial were obtained with 20 individual data for each feed.

Dry matter and fat were analysed in feeds and feces in order to determine dry matter and fat digestibility according to the well known procedure commonly used in animal nutrition.

The results were submitted to variance analysis and Duncan's comparison of the means.

The results obtained (table 1) show that the SP 343 enzyme improved dry matter and fat digestibility but that the lipase (Lipozyme 10000 L) was not able to improve digestibility of these components.

Table 1

Lots	Feed	Dry matter Digestibility (%)	Fat Digestibility (%)
1	Basal	63.0±4.2 A	43.1±17.1 A
2	Basal +Enzyme 200 g/t	67.8±3.9 B	54.8±14.7 B
3	Basal +Enzyme 1000 g/t	68.7±3.4 B	58.0±11.5 B
4	Basal +Lipase	59.7±5.3 A	36.6±17.4 A
Variance analysis		$p \leq 0.001$	$p \leq 0.001$

(In a same column, A and B means a significant difference ($p \leq 0.001$) with Duncan's test).

EXAMPLE 2

320 chicken were taken, at 8 days of age, from a flock raised in an area where malabsorption syndrome was endemic.

The birds were divided in 4 lots of 80 birds.

The birds were raised in individual cages as previously described in Example 1.

Each lot of birds received one of these 4 experimental feeds, for three weeks (from 8 to 29 days of age):

1) Basal feed
2) Basal feed supplemented with enzyme SP 343 at 200 g/ton
3) Basal feed supplemented with enzyme SP 343 at 1000 g/ton
4) Basal feed supplemented with Lipozyme 10000 L at 50 g/ton

Body weight gain, feed intake and feed:gain ratio were measured for each bird from 8 to 29 days of age.

Results were submitted to statistical analysis as previously described.

The results (table 2) show that birds consuming the basal feed had high feed:gain ratio. The SP 343 enzyme improved growth, and decreased feed intake and feed:gain ratio significantly. The lipase enzyme added at a dose rate in excess of the lipase side activity present in SP 343 did not significantly change the feed:gain ratio.

Table 2

Lots	Feeds	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	Feed:gain ratio
1	Basal	142.4	925.8a (100.0)	783.4a (100.0)	1487.0a (100.0)	1.904A (100.0)
2	Basal +Enzyme 200 g/t	142.9	984.8ab (102.6)	806.9ab (103.0)	1479.8ab (99.5)	1.842B (96.7)
3	Basal +Enzyme 1000 g/t	143.0	960.7b (103.8)	817.7b (104.4)	1439.3b (96.8)	1.767C (92.8)
4	Basal +Lipase 50 g/ton	142.4	940.6ab (101.6)	798.2ab (101.9)	1504b (101.2)	1.892A (99.4)
Variance analysis		p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.001

In a same column, Duncan's test:

a, b : p≤0.05

A, B, C : p≤0.001

EXAMPLE 3

320 broilers were taken, at 20 days of age, from a farm located in an area where malabsorption syndrome was endemic.

After being weighed, they were distributed in 4 lots of 4 replications of 20 birds for each replication.

The birds were raised on floor pen with wood shaving litter (2 m_2/pen).

Each lot of birds received one of these 4 experimental feeds, from 20 to 41 days of age (21 days of experiment):

1) Basal feed 1 - maize base
2) Basal feed 1 supplemented with enzyme SP 343 at 1000 g/ton
3) Basal feed 2 - wheat base
4) Basal feed 2 supplemented with enzyme SP 343 at 1000 g/ton

The xanthophyll composition of the 2 basal feeds are on table 3.

Table 3

	Maize base	Wheat base
Lutein (ppm)	41.80	42.90
Zeaxanthin (ppm)	13.00	12.00
Canthaxanthin (ppm)	0.92	1.03

On the 14th day of the experiment, blood plasma was collected from 12 birds of each treatment (3 birds of each replication) for measuring the

optical density by spectrophotometry (with a spectrophotometer Beckman DU 64) at 450 nm. The optical density of plasma reflects the absorbed xanthophyll content of the blood.

Weight gain, feed intake and feed:gain ratio of the birds were also measured for the 21 days of experiment.

After the 21 days of experiment all the birds were slaughtered in an industrial abattoir. After cooling for 24 hours, the pigmentation of the carcasses was ranked by 2 well trained quality controllers of the abattoir. Each bird was given a score:

1: badly pigmented
2: moderately pigmented
3: well pigmented

The average score was calculated for each treatment.

RESULTS

Optical Density of Plasma

Table 4

Lots	Feeds	Optical Density	Coefficient of Variation (%)
1	Maize base	0.441 ± 0.173	39.3
2	Maize base + enzyme	0.605 ± 0.163	27.3
3	Wheat base	0.407 ± 0.173	43.7
4	Wheat base + enzyme	0.615 ± 0.117	19.0
Variance analysis		$p \leq 0.001$	

Table 4 shows that optical density was significantly increased by the enzyme. There were therefore more xanthophylls in the blood.

Coefficient of variation of optical density was also lower with birds receiving enzyme: The xanthophyll contents of the blood were more homogenous with enzyme supplemented feeds than with control feeds.

Pigmentation of Carcasses

Table 5 shows that the pigmentation score of carcasses was increased by the enzyme. In basal feeds, 2 to 5 percents of birds were scored as badly pigmented, while there was no such scored bird with enzyme.

Table 5

Lots	Feeds	Score	Contr.1 %birds	Score	Contr.2 %birds	Score	Average Score
1	Maize base	3 2 1	20 77 3	2.17	20 78 2	2.18	2.175
2	Maize base + Enzyme	3 2 1	31 69 0	2.31	25 75 0	2.25	2.280
3	Wheat base	3 2 1	14 84 2	2.12	15 80 5	2.10	2.110
4	Wheat base + Enzyme	3 2 1	29 71 0	2.29	30 70 0	2.30	2.295

Zootechnical Performances

Table 6

Lots	1	2	3	4
Feeds	Maize base	Maize base + enzyme	Wheat base	Wheat base + enzyme
Live weight (g)				
initial	510±5	512±8	520±1	1517±15
final	1651±4	1661±28	1656±39	1632±71
Weight gain(g)	1141±8	1150±25	1136±32	1114±57
Feed intake(g)	2570±79	2551±10	2608±123	2438±135
Feed:gain ratio	2.253±0.078	2.219±0.052	2.296±0.072	2.188±0.039

Table 6 shows that the enzyme:

- Improved slightly growth of birds with maize based feed.
- Decreased feed intake and feed:gain ratio for both kinds of feeds.

EXAMPLE 4

Digestibility Trials

Cecectomized cockerels are divided at random into groups of 6 birds. A basal wheat based feed is used supplemented with enzyme at the rate of 1 kg per ton. The birds are starved for 30 hours and then each fed 80 g of feed. Faeces are collected from each bird and then pooled for each treatment

group. The pooled samples are oven dried at 70°C and then analysed by the current EEC methods except for fat which is analysed by acid hydrolysis.

The results in table 7a and 7b show that the specific cellulase/xylanase combination found in SP 343 and SP 431 are able to improve fat digestibility in the cecectomized cockerel but that lipase or standard cellulase from Tricoderma sp is not able to improve digestibility.

Table 7a

Enzyme	True Digestibility, % Dry matter	Fat
Control	72.95	87.78
SP 343	72.87	90.98
SP 431	74.04	91.59
Lipolase	71.62	87.98

Table 7b

Enzyme	True Digestibility, % Dry matter	Fat
Control	72.28	87.16
SP 343	72.26	88.52
Celluclast	72.36	86.73

EXAMPLE 5

Comparison of the Effect of a Conventional Cellulase and Growth Promoting Agent

468 days old chickens were placed into 18 pens each containing 26 birds (13 male and 13 female). The pens were randomly divided into 3 groups which received the basal feed (Table 8) with the following supplements:

Group A :	No supplement
Group B :	Celluclast 1.5 L at 1000 g/ton feed
Group C :	Virginianycin at 90 ppm

Feed and water were supplied ad lib and body weight gain, feed intake and feed conversion were measured over the 47 day period of the trial. The results were submitted to statistical analysis as previously described.

The results show (Table 9) that addition of a traditional cellulase preparation from Trichoderma reesei (Celluclast 1.5 L) did not significantly influence the feed intake or weight gain of broiler chickens fed a wheat based diet.

Table 8

Feed Composition for Example 5

	0-28 days	29-49 days
Wheat	58.0%	71.0%
Soy bean meal	28.5%	15.0%
Meat & bone meal	5.0%	5.0%
Corn gluten	3.0%	3.0%
Fat	2.0%	2.0%
Trace minerals/vitamins	3.5%	4.0%
Crude Protein	23.8%	19.0%
Energy (Kcal/kg)	2892	2992

Table 9

Feeding Results

	A	B	C
Overall weight gain (kg)	2.09	2.11	2.18[a]
Overall feed intake (kg)	4.25	4.35	4.43[a]
FCR	2.05	2.02	2.03

Values with a superscript a) in a single row are significantly different from the control ($p \leq 0.05$)

EXAMPLE 6

Comparison of the effect of a traditional cellulase (SP 346, commercial cellulase from *Trichoderma reesei*) with SP 343 (*Humicola insolens* cellulase/xylanase, DSM 1800), and SP 431 (*Trichoderma longibrachiatum* xylanase, NRRL 11460)

350 chickens were taken from a commercial flock, at 8 days of age and divided into 7 lots of 50 birds.

The birds were raised in individual cages as previously described in Example 1.

The birds were fed on a basal wheat based feed (Table 10) supplemented with enzymes according to the following scheme :

SP 346	0.02% and 0.1%
SP 343	0.02% and 0.1%
SP 431	0.01% and 0.025%

Table 10

COMPONENT	% IN THE DIET
Wheat	61.40
Soybean meal 46% C.P.	22.00
Fishmeal 65% C.P.	5.00
Meat and Bone meal 50% C.P.	3.30
Calcium carbonate	0.61
Salt	0.28
DL-Methionine 98%	0.17
L-lysine 78%	0.04
Animal fat	6.20
Vitamin/Trace Mineral premix*	0.50
Chromium oxide	0.50

Analysis

A.M.E. (Kcal/kg)	3,120
Protein (%)	22.15
Sulphur amino acids (%)	0.92
Lysine (%)	1.20
Fat (%)	7.85
Crude fiber (%)	3.00
Calcium (%)	0.95
Available Phosphorous (%)	0.40

* Supplies : monensin 100 ppm, bacitracin 50 ppm

Body weight gain, feed intake and feed:gain ratio were measured for each bird from 8 to 22 days of age. A balance experiment was also carried out in which the apparent digestibility of Dry Matter, Fat and Crude Fiber were determined.

The results (Table 11) show that the performance of those chickens receiving the conventional cellulase (SP 346) was not significantly different to that of the control whereas those birds reciving SP 343 or the higher dose of SP 431 had a significantly improved Feed:Gain ratio.

Table 11

	TREATMENTS						
	Control	0.02% SP 343	0.1% SP 343	0.02% SP 346	0.1% SP 346	0.01% SP 431	0.025% SP 431
Weight gain (g)	519.88	525.20	514.87	523.59	508.48	520.79	528.19
(S.D.)	36.72	42.81	38.85	49.06	43.61	38.06	44.23
Feed intake (g)	775.38	765.86	752.59	773.15	754.10	763.94	765.58
(S.D.)	59.60	58.99	65.54	67.31	61.72	59.26	59.56
Feed:Gain ratio	1.493	1.460	1.462	1.479	1.485	1.468	1.452
(S.D.)	0.070	0.066	0.063	0.070	0.068	0.063	0.077
	(100)	(97.8)	(97.9)	(99.1)	(99.5)	(98.3)	(97.2)
	a	bc	bc	abc	ab	abc	c

Values for Feed:Gain ratio with a different letter are significantly different at a probability value $p<0.5$.

The results in Table 12 show that again only the SP 343 and SP 431 enzymes had a positive effect on the apparent digestibility of Dry Matter, Fat and Crude Fiber.

Apparent Digestibility (%)	TREATMENTS						
	Control	0.02% SP 343	0.1% SP 343	0.02% SP 346	0.1% SP 346	0.01% SP 431	0.025% SP 431
Dry matter	73.31	76.16	75.31	74.20	72.64	75.12	74.07
(S.D.)	1.75	1.41	1.57	0.70	1.31	1.23	1.29
	CD	A	AB	BC	D	AB	BC
Fat	82.18	84.19	85.05	83.28	81.34	84.32	85.08
(S.D.)	59.60	58.99	65.54	67.31	61.72	59.26	59.56
	bc	ab	a	abc	c	ab	a
Crude Fiber	6.13	14.22	9.28	5.56	4.04	11.49	6.48
(S.D.)	5.64	4.82	4.52	3.10	4.56	6.10	7.87
	C	A	ABC	C	C	AB	BC

Values for Apparent Dry Matter or Crude Fiber Digestibility with a different letter are significantly different at a probability value $p<0.001$.

Values for Apparent fat Digestibility with a different letter are significantly different at a probability value $p<0.05$.

CLAIMS

1. Feed additive for alleviation of the malabsorption syndrome in poultry, characterized by the fact that it comprises a cellulase and a xylanase producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma longibrachiatum* (NRRL 11460).

2. Poultry feed, characterized by the fact that the poultry feed is composed of a normal poultry feed, to which a feed additive according to Claim 1 has been added.

3. Method of poultry feeding characterized by the fact that the poultry is the poultry feed according to Claim 2.

4. Method according to Claim 3, characterized by the fact that the feed additive according to Claim 1 is added to the drinking water for the poultry.

5. Method according to Claim 3, characterized by the fact that the feed additive according to Claim 1 as a solid material is added to the normal poultry feed.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 90/00256

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 23 K 1/18, 1/165

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 23 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Med. Fac. Landbouww. Rijksuniv. Gent, Vol. 53, No. 4a,1988 W.D. COWAN: "SELECTION AND APPLICATION OF ENZYMES TO IMPROVE UTILIZATION OF ANIMAL FEEDS", see page 1773 - page 1783 --	1-2
X	US, A, 3462275 (W.D. BELLAMY) 19 August 1969, see column 1, line 19 - line 21; column 4, line 67 --	1-2
X	Derwent's abstract, No. 78-649 21A/36, SU 577 230, publ. week 7836 MOSC FOOD IND TECHN; BIOTECH RES INST --	1-2
Y	EP, A2, 0280226 (SUOMEN SOKERI OY) 31 August 1988, see page 5, line 4 - line 46; claim 5 --	1-2

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th January 1991	1991 -01- 08
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Inga-Karin Petersson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Derwent's abstract No. 36 259 K/15, SU 933 063, publ. week 8315 POULTRY RES INST	1-2
Y	Chemical Abstracts, volume 99, no. 23, 5 December 1983, (Columbus, Ohio, US), Isshiki, Y. et al.: "Effect of pectinase, xylanase and cellulase supplements on the utilisation of feed in chicks ", see pages 652-653, abstract 193693d, & Nippon Kakin Gakkaishi 1983, 20(4), 237- 243	1-2
Y	Chemical Abstracts, volume 96, no. 13, 29 March 1982, (Columbus, Ohio, US), Hayashida, S. et al.: "Production of thermostable cellulases and raw starch-digesting amylases by fungi ", see page 387, abstract 100626j, & Adv.Biotechnol.,(Proc.Int.Ferment.Symp.) 1981, 3 , 271- 276	1-2
Y	Chemical Abstracts, volume 98, no. 15, 11 April 1983, (Columbus, Ohio, US), Sandhu, D.K.: "Production of cellulase, xylanase and pectinase by Trichoderma longibrachiatum on different substrates ", see page 483, abstract 124113, & Trans.Br.Mycol.Soc. 1982, 79(3), 409- 413	1-2
A	Chemical Abstracts, volume 76, no. 7, 14 February 1972, (Columbus, Ohio, US), Bakai, S.M.: "Use of fungal cellulase in the peparation of feeds ", see page 228, abstract 33005c, & Fermenty Nar. Khoz. Med. 1971, 155- 158	1-2

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 3-5, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the animal body by therapy [PCT Rule 39(iv)].

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 90/00256

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-11-28
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 3462275	69-08-19	BE-A-	727641	69-07-01
		DE-A-B-	1904239	69-09-04
		GB-A-	1245434	71-09-08
		NL-A-	6901528	69-08-04
EP-A2- 0280226	88-08-31	JP-A-	63291543	88-11-29